NanoEnTek

CE 0123 IVD

FREND™ PSA Plus
Prostate Specific Antigen

Caution:

The sale and distribution of this device is restricted by United States federal law to, by, or on the order of a physician. In addition, the use of this device is restricted to, by, or on the order of a physician. Because of differences in reagent specificity and assay methods, the concentration of PSA in a given specimen may vary with devices from different manufacturers. Values obtained with different assay methods cannot be used interchangeably. It is mandatory that results reported by the laboratory to the physician include the identity of the assay used. If the assay method for PSA is changed during the course of monitoring patients with serial PSA levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

1. Name and Intended Use

The NanoEnTek FREND™ PSA Plus is designed for *in vitro* DIAGNOSTIC USE ONLY for the quantitative measurement of total Prostate Specific Antigen (PSA) in human serum, heparinized plasma, and EDTA plasma using the FREND™ System. This device is indicated for the serial measurement of total PSA in serum, heparinized plasma and EDTA plasma to be used as an aid in the management of patients with prostate cancer.

2. Summary and Explanation of Test

Prostate-specific antigen (PSA) is a single-chain glycoprotein with molecular weight of 34 kilodaltons.[1, 2, 3] As a serine protease with chymotrypsin-like activity, PSA belongs to the kallikrein family. In blood, PSA exists as a free or complex form with protease inhibitors such as α-1-antichymotrypsin (ACT). Total PSA represents the sum of both free and complex forms.[4] PSA is uniquely associated with prostate tissues from normal, inflamed or cancerous stages. Elevated PSA in serum or plasma is found in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory tissues. Studies on a variety of PSA methods have shown that PSA can be useful as an indicator for the diagnosis and management of prostate cancer.[5]

PSA has been found in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid as well as in seminal fluid.[6] PSA is not found in any other tissue in men, and it is not produced by cancers originating in the lung, colon, rectum, stomach, pancreas or thyroid.[7] Though increased concentrations of PSA are found in the serum of patients with benign prostate hyperplasia (BPH), prostatitis and prostate infections and inflammation, they are also found in patients with cancer of the prostate.[8, 9] PSA measurement is an essential tool in assessing the status of disease in patients with prostate cancer when serial samples are measured over time.[10] The clinical value realized by monitoring tPSA concentrations in patients with prostate cancer regardless of the treatment regimen is well known.[11] Since the mid-1980's, there has been a growing body of literature concerning the utility of Prostate Specific Antigen (PSA) for both the monitoring and detection of prostate cancer (CaP).

3. Principle of the Assay

The FREND™ PSA Plus is a rapid quantitative "sandwich" immunoassay using fluorescent nanoparticles which measures the concentration of total PSA. Thirty µL of patient serum or plasma (heparin or EDTA only) is manually presented to the inlet on the individual single-unit test cartridge where it is mixed with fluorescent nano-particles conjugated with PSA antibodies. PSA molecules in the specimen bind to conjugated antibodies to form immune complexes which then move by capillary action through the reagent cartridge channel to the detection area. When the specimen reaches the test zone, it hydrates dried solid-phase anti-PSA antibodies. PSA-fluorescent particle-immune complexes in the specimen are grabbed by the capture antibodies to form sandwich immune-complexes. The residual PSA-unbound fluorescent nano-particles conjugated with PSA-antibodies pass through the test zone and bind to PSA antigens in the reference zone. As the sample moves forward to the waste reservoir, non-specific binding components are washed away. The intensity of fluorescence measured by a light source (laser) is proportional to the amount of total PSA in the original sample. The result is calculated using information stored on the lot specific FREND™ PSA Plus Code chip and then is displayed on the FREND™ System screen. A hard copy printout can be obtained if desired. A ratio calculated between the Reference zone and the Test zone corrects for test-to-test variations.

Total PSA concentration in a sample analyzed with the FREND™ PSA Plus on the FREND™ System correlates directly with the fluorescence intensity - the higher the tPSA concentration, the greater the fluorescence. The FREND™ PSA Plus has a measuring range determined as 0.1 ng/mL to 25.0 ng/mL.

The FREND™ PSA Plus uses single-use transparent plastic cartridges in which all required reagents are stored within the cartridge itself. All that is added by the user is a 30 µL test sample. The cartridge is inserted into the FREND™ System in a prescribed fashion indicated with a black arrow on the cartridge. The reaction is read multiple times as the sample moves via capillary action through the cartridge. This type of assay system is sometimes referred to as one which incorporates laminar flow.

4. Material Provided (FREND™ PSA Plus) * Catalog Number : FRPS 025

FREND™ PSA Plus cartridges	25
Disposable pipette tips (micro-pipettor provided)	30
FREND™ PSA Plus Code chip	1
FREND™ PSA Plus Package Insert	1

One Cartridge contains:
Monoclonal anti-PSA1 ---------- 48 ± 9.6 ng
Monoclonal anti-PSA2 ---------- 144 ± 28.8 ng
Fluorescent particle ---------- 2.4 ± 0.48 µg

5. Materials Required But Not Provided

The following materials are not provided with the reagent but are required to perform Prostate Specific Antigen analysis using the FREND™ PSA Plus on the FREND™ System. They are available separately from NanoEnTek.

Materials	Cat. No.
FREND™ System	F10

6. Warnings and Precautions

- The FREND™ PSA Plus cartridges are intended for *in vitro* diagnostic use only.
- PSA Plus cartridges are only to be used on the NanoEnTek FREND™ System.
- Allow cartridges to come to room temperature for 15 ~ 30 minutes prior to use.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
- Inaccurate results are possible if the sample used is contaminated in any way.
- Using specimens containing clotted fibrin could result in erroneous results.
- Over or under loading the cartridge with sample may result in inaccurate results.
- Cartridges should not be frozen.

Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human materials, please use standard laboratory safety procedures when handling all specimens and controls.

- Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package Insert and User Manual.
- PSA Plus cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Keep the cartridge sealed in the pouch until just ready for use.
- Use the cartridge immediately after opening its pouch.
- Wear disposable gloves when handling the cartridges and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- For professional use only.

PSA Plus has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with PSA concentrations between 25 and 1,200 ng/mL will read > 25 ng/mL. The "hook effect" phenomenon may occur only at PSA concentrations > 1,200 ng/mL.

7. Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Cartridge stability has been demonstrated for twelve months from the date of manufacture.

The expiration date is clearly indicated on the product box and the cartridges.

Materials	Cat. No.
Refrigerator Temperature (2 ~ 8° C) : PSA Plus cartridges	FRPS 025
Room Temperature (18 ~ 25° C): Pipette Tips	None

8. Specimen Collection and Handling

Serum or plasma (heparinized or EDTA only) is required for the assay. Citrated plasma SHOULD NOT BE USED.

No special patient preparation is necessary. To use serum, a blood sample is collected aseptically without additives by venous puncture. After allowing the sample to clot for 30 minutes at room temperature, the collection tube should be centrifuged for 10 minutes at 3,000 rpm.

For heparinized or EDTA plasma, a venous blood sample is collected aseptically with the designated additive. The plasma should be separated from the packed cells as soon as possible.

Prostatic manipulation has been shown to affect the PSA results so samples should be drawn before any prostatic procedures such as DRE, prostatic massage and TRUS are performed.

Samples may be stored at 2 ~ 8 °C for up to 6 hours prior to analysis. If the analysis is scheduled to be done at some later time, the sample should be stored frozen at -20° C or below for future use.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter such as fibrin clots or strands should be centrifuged before being tested. Prior to assay, slowly bring frozen samples to room temperature (18 ~ 25 °C) and mix gently but thoroughly before testing.

9. Procedure

1) Reagent Preparation

- **Cartridges**

  There is no reagent preparation required to measure tPSA using the FREND™ PSA Plus cartridge on the FREND™ System. However, the cartridges needed for a particular run should be removed from the refrigerator and allowed to reach room temperature for 15 ~ 30 minutes before they are used.

2) Calibration

The calibrators used during the cartridge manufacture process to create the information placed electronically on the FREND™ PSA Plus Code chip are prepared gravimetrically and are compared to international reference standards (WHO International Prostate Specific Antigen (90:10) NIBSC code: 96/670). However, for the end user, there is no need for calibration as is generally performed on other automated laboratory equipment. All calibration statistics and information have been electronically stored on the FREND™ PSA Plus Code chip included in each box of FREND™ PSA Plus cartridge. The FREND™ PSA Plus Code chip is specific for that manufactured lot of FREND™ PSA Plus.

The appropriateness of the calibration information should always be checked by running sufficient external quality control materials as samples to verify that the results obtained for tPSA on the FREND™ System using the FREND™ PSA Plus cartridges of a particular lot meet the laboratory criterion for acceptability.

- **FREND™ PSA Plus Code chip Installation**

  Please refer to the FREND™ System User Manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions follow here:

  (1) Insert the FREND™ System electrical cord into an appropriate outlet.
  (2) Insert the Code chip into the Code chip slot at the rear of the FREND™ System following the arrows.
  (3) Press the 'Setup' button on the 'Main' screen.
  (4) Press the 'Code chip' button on the 'Setup' screen.
  (5) The information embedded on the FREND™ PSA Plus Code chip is automatically saved on the FREND™ System.
  (6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.
  (7) Press the 'Item' button on the 'Setup' screen.
  (8) Check the FREND™ PSA Plus cartridge lot number and the installation date of the Code chip.
  (9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

3) Quality Control

- **FREND System QC Cartridge**

  FREND™ QC Cartridge contains multiple controls to check optic part of the system. By testing QC Cartridge, part of analytical components of the system of (1) laser power, (2) alignment, and (3) mechanical integrity are confirmed.
  For each day of patient testing, perform QC Cartridge testing. Refer to the Quality Control Procedures section in the User Manual of FREND™ System. In brief, perform QC Cartridge testing for the following conditions:

(1) Upon initial setup of the system.
(2) Each day of patient testing.
(3) When the system has been transported or moved.
(4) Whenever there is uncertainty about the performance of the system.
(5) Whenever required by your laboratory's quality control requirements.

- **Internal procedural controls**

FREND™ PSA Plus test cartridge contains built in control features. Fluorescence signal in the Reference Zone of each cartridge shows: (1) that enough volume is added, (2) that proper flow is obtained, and 3) that the antibody is reactive. If this reference zone signal is missing or lower than threshold, the FREND™ system consider it as an incorrect or failed test, not producing a test result but an error message. In addition, with each cartridge run, the system monitors, in part, for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

- **External quality control testing**

Commercially available controls from a variety of manufacturers are available that contain tPSA as a measured analyte. It is recommended that a minimum of at least two (2) levels of controls be run at least once per month or once for each new lot, whichever comes earlier. However, Controls should be run with a minimum frequency, depending on number of tests run in the laboratory. Each laboratory should establish its own criteria based on the following parameters:

(1) Each new lot
(2) Each new shipment (even if from the same lot previously received)
(3) Each new operator (an individual who has not run the tests for at least two weeks)
(4) Monthly, as a continued check on storage conditions
(5) Whenever problems (storage, operator, or other) are identified
(6) Or other times as required by your laboratory's standard QC procedures

Individual laboratory policy will dictate exactly which control materials and lot numbers should be run, the frequency with which controls are to be tested, criteria for acceptance of the results and required corrective action to be taken if results do not meet laboratory criteria. If any external quality control sample values are out of the acceptable range, it will be necessary to investigate the problem before reporting patient results to assure there is not an instrument or software malfunction. Do not assay patient samples on the FREND™ System using the FREND™ PSA Plus if quality control results do not give expected values. Refer to your laboratory policies on how to determine acceptability of external control material results.

Each laboratory operates under a different set of regulations. Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to whom the laboratory is responsible.

4) Specimen Processing

- **Preparation**

  Remove from the refrigerator sufficient cartridges of FREND™ PSA Plus to test the number of patient samples and required external quality control materials. Allow the cartridges to come to room temperature for 15 – 30 minutes prior to the start of the testing sequence.

  If using refrigerated patient samples, remove those from the refrigerator and allow to them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing.

  There are no other reagents or sample preparations necessary.

- **Assay Procedure**

  (1) Prepare the FREND™ PSA Plus and specimen.
  (2) Record the Sample ID on the cartridge in the designated area.
  (3) Drop the sample (30 µL) into the sample inlet on the cartridge using a calibrated micro-pipette with a fresh pipette tip.
  (4) Press the 'Test' button on the 'Main' screen of the FREND™ System.

(5) The screen of FREND™ System to the Patient ID screen automatically.
(6) Type the Patient ID and press the 'Enter' button to begin the test.
(7) Insert the cartridge into the cartridge slot using the cartridge arrows as a guide.

⚠ **Caution:** Please check the direction of the cartridge before insertion and assure the insertion is complete.

(8) When the reaction in the cartridge is complete within 4minutes, the FREND™ System will automatically begin the reading process.
(9) When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

⚠ **Caution:** Do not remove power from the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.

(10) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.
(11) For more detailed instructions, please refer to the 'FREND™ System User Manual.'

10. Procedural Notes

If a specimen Prostate Specific Antigen concentration is found to be greater than the linearity limit of the assay of 25.0 ng/mL and a definitive result is required, the specimen should be diluted with female sera that has been previously measured on the FREND™ PSA Plus and found to contain < 0.1 ng/mL tPSA and then re-assayed according to the Assay Procedure. The recommended dilution for samples with an initial result of >25.0 ng/mL is 1:10 or 1:50. It is desirable to dilute the sample so that the diluted sample reads between 2 and 20 ng/mL. Dilutions must be made manually and the final result on the diluted sample calculated manually by multiplying the result obtained on the diluted sample by the dilution factor.

11. Calculation of Results

The FREND™ System performs all sample and cartridge handling operations automatically within the cartridge once the sample has been manually added to the sample well in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process, blank readings are subtracted after which the net rate is automatically converted to total Prostate Specific Antigen concentration in ng/mL based upon information stored on the PSA Code chip. This result is then output on the screen and to the optional printer. It is also stored in memory on the FREND™ System.

Screen Displays for Various Concentration Scenarios

Displayed result	Description
List Print Date/Time : 2012-1-13 10:55 AM User ID : Nano Patient ID : T-01 PSA < 0.10 ng/mL	PSA concentration Less than 0.10 ng/mL
List Print Date/Time : 2012-1-17 11:55 AM User ID : Nano Patient ID : T10 PSA 9.73 ng/mL	PSA concentration Not less than 0.10 ng/mL and not higher than 25.00 ng/mL
List Print Date/Time : 2012-1-13 11:30 AM User ID : Nano Patient ID : T-04 PSA > 25.00 ng/mL	PSA concentration Higher than 25.00 ng/mL

12. Limitations of the Procedure

When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, medical history, therapy, etc.).

The FREND™ System, paired with a FREND™ PSA Plus cartridge, is programmed to report 25.0 ng/mL, as the highest concentration of PSA measurable without dilution. The lowest measurable concentration is 0.1 ng/mL, the assay sensitivity limit.

Heterophilic antibodies in a sample have the potential to cause interference in immunoassay systems.[12,13] Infrequently, PSA levels may appear elevated due to heterophilic antibodies present in the patient's serum or plasma or to nonspecific protein binding. If the PSA level is inconsistent with clinical evidence, additional PSA testing is suggested to confirm the results.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where interference with the lateral flow of the sample in the cartridge may occur.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated or decreased PSA values.

Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.[14]

The concentration of tPSA in a given sample determined with assays from different manufacturers can vary due to differences in assay methods, calibration, and reagent specificity.[15]

Please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Clinical results must be interpreted with regard to medications administered to the patient.[14]

The ability of the assay to detect both free and complexed forms of total PSA (free PSA complexed with alpha-1-antichymotrypsin) on an equal molar basis (equimolarity) has not been established.

13. Expected Values

As with every clinical diagnostic test, a reference interval corresponding to the characteristics of the population being tested should be determined by each laboratory. The FREND™ PSA Plus on the FREND™ System is to be used on serial blood samples to manage patients with prostate cancer.

Testing of ambulatory male subjects fifty years old and older who reported themselves as healthy without any known illnesses, diseases or conditions was performed using both the FREND™ PSA Plus on the FREND™ System and another commercially available PSA method. The currently accepted reference interval for tPSA of up to 4.0 ng/mL was validated for both systems.

As is true for all PSA methods, no tPSA results can be interpreted as being definitive for the presence or absence of prostate cancer. Patients with levels of PSA within the reference interval found in apparently healthy subjects may have prostate cancer; patients with levels exceeding those in the reference interval may be prostate cancer free. Results from the FREND™ PSA Plus on the FREND™ System should be interpreted in the light of other clinical findings and diagnostic procedures such as DRE, various imaging studies, etc. since certain treatments can cause PSA values to decrease by virtue of the treatment while the cancer is still progressing.

14. Reference Ranges

The interval given here was determined in serum samples from 196 apparently healthy male subjects from the age of 50 ~ 71 years.

Category	Men
Number of Samples (n)	196
Reference Interval	0 ~ 4.0 ng/mL

In this study, greater than 99% of the healthy subjects had serum PSA concentrations less than or equal to 4.0 ng/mL by FREND™ PSA Plus on both the FREND™ System and another commercially available tPSA fluorescent assay. Results of this study are shown below:

Category	Other FDA Cleared PSA	PSA Plus on FREND™
Number of Samples (n)	196	196
Mean (x)	0.71 ng/mL	0.83 ng/mL
SD	0.38 ng/mL	0.43 ng/mL
Range of Values	0.00 ~ 2.03 ng/mL	0.02 ~ 2.69 ng/mL
Median	0.66 ng/mL	0.77 ng/mL

Expected Values for Management of Patients with Prostate Cancer

Distribution of Serum FREND™ PSA Plus Concentrations
Healthy, Benign and Various Malignant Disease States

	N	0 – 4.0 ng/mL	4.1 – 10.0 ng/mL	10.1 – 20.0 ng/mL	20.1 – 40.0 ng/mL	>40.0 ng/mL
Healthy Subjects	196					
Men ≥ 50 yrs.	196	100%	0%	0%	0%	0%
Benign Disease/Cond*	**410**					
Benign Prostate	104	56.73%	25.96%	11.54%	3.85%	1.92%
Diabetes	97	95.88%	3.09%	1.03%	0.00%	0.00%
HTN/Heart Disease	102	95.10%	4.90%	0.00%	0.00%	0.00%
Benign GI	107	94.4%	4.67%	0.00%	0.93%	0.00%
Malignant Diseases*	**302**					
Prostate Cancer**	85	40.00%	38.82%	12.95%	2.35%	5.88%
Gleason Score 5-6	43	51.16%	44.19%	2.38%	2.38%	0%
Gleason Score 7	31	35.48%	38.72%	19.35%	0%	6.45%
Gleason Score 8-9	11	9.09%	18.18%	36.36%	9.09%	27.27%
Lung/Liver Cancer	52	98.08%	0%	1.92%	0%	0%
GB,Gastric,Pancreatic	31	100%	0%	0%	0%	0%
Colorectal Cancer	89	94.38%	4.49%	1.13%	0%	0%
Other Cancers	45	97.78%	2.22%	0%	0%	0%
TOTAL Subjects	**908**					

* Treated and untreated subjects
** Serial samples are not included in this cohort.

15. Performance Characteristics

Performance characteristics were evaluated for the FREND™ PSA Plus as follows:

1) Accuracy

1a) Recovery

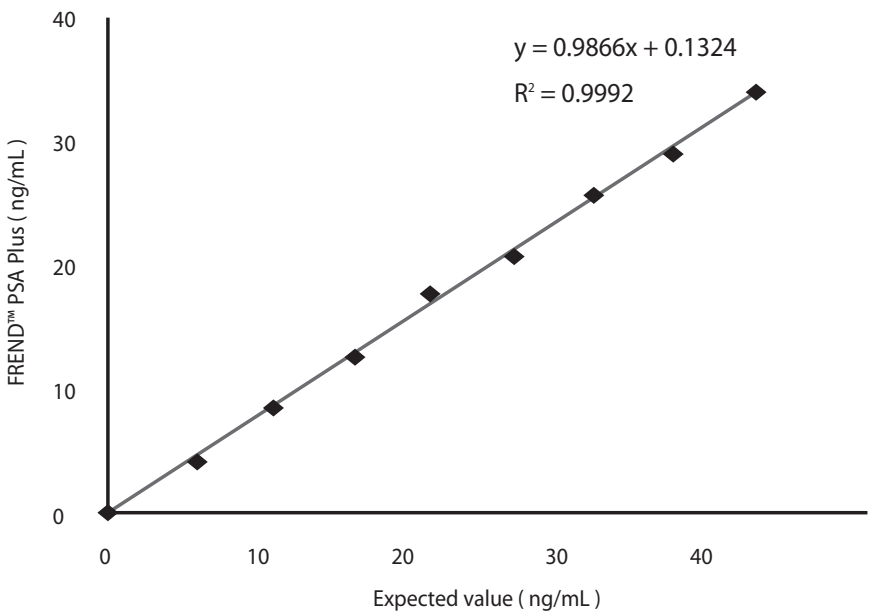
Known samples of PSA were added to a female serum specimen (0.01 ng/mL). The concentration of PSA was determined before and after the addition of the exogenous PSA and the percent recovery was calculated.

Spiked Recovery - FREND™ PSA Plus

Concentration Added (ng/mL)	Observed Concentration (ng/mL)	Recovery (%)
1.08	1.06	98.3
	1.09	100.7
	1.04	96.7
4.34	4.42	101.8
	4.35	100.3
	4.27	98.4
12.81	13.58	106.0
	12.04	94.0
	11.82	92.3
25.53	24.26	95.0
	24.67	96.6
	26.90	105.4

1b) Dilution Linearity:

Specimens from a high concentration pool (34 ng/mL tPSA) were diluted with a low concentration pool following instructions in the CLSI EP6-A document. Correlation with the expected linearity showed $R^2 = 0.9992$.



Dilution Linearity - FREND™ PSA Plus

Dilution Linearity Data - FREND™ PSA Plus

No.	Blank	1	2	3	4	5	6	7	High
Dilution	**0.000**	**0.125**	**0.250**	**0.375**	**0.500**	**0.625**	**0.750**	**0.875**	**1.000**
TEST 1	0.00	4.63	8.85	13.30	18.34	21.44	23.78	28.04	39.98
TEST 2	0.00	4.65	8.65	12.33	16.89	22.49	25.42	34.33	35.22
TEST 3	0.10	4.08	9.24	12.71	18.22	19.39	27.13	27.12	31.74
TEST 4	0.00	4.27	8.18	13.91	16.07	19.32	26.88	29.51	27.43
TEST 5	0.00	4.59	8.67	12.61	17.56	20.67	24.91	27.36	28.32
TEST 6	0.10	4.26	7.74	11.58	18.57	22.78	25.93	26.38	40.09
MEAN (ng/mL)	**0.033**	**4.413**	**8.555**	**12.740**	**17.608**	**21.015**	**25.675**	**28.790**	**33.797**
SD		0.241	0.526	0.802	0.972	1.490	1.255	2.913	5.561
CV(%)		5.5	6.1	6.3	5.5	7.1	4.9	10.1	16.5
Expected Value	**0.0**	**4.3**	**8.0**	**12.8**	**17.0**	**21.3**	**25.5**	**29.8**	**34.0**
% Recovery		**103.8**	**106.9**	**99.9**	**103.6**	**98.9**	**100.7**	**96.8**	**99.4**

1c) Comparative Analysis:

A group of well-characterized serum samples collected with IRB oversight from subjects with pathology-verified prostate cancer and stored at -70° C under monitored conditions were analyzed for total PSA. Results from the FREND™ PSA Plus on the FREND™ System (y) were compared to those obtained using the TOSOH ST AIA-PACK PA assay (x). A total of 160 unique samples were analyzed in the study, however, only samples with tPSA results within the linearity of the FREND™ PSA Plus (up to 25.0 ng/mL; (n=143)) were used in the comparative analysis.

Slope: 0.9192 (95% CI: 0.8369; 1.0014)
Intercept: -0.01179 (95% CI: -0.2763; 0.2527)
Correlation Coefficient (R) = 0.9671 (95% CI: 0.9545 ~ 0.9763)
Number of Samples = 143
Range of FREND™ PSA Plus values: 0.04 ~ 29.99 ng/mL
Range of ST AIA-PACK PA values: 0.00 ~ 25.86 ng/mL

2) Precision

2a) Precision Testing Single Lot and Single Site

Precision was determined as described in the CLSI protocol EP5-A. Four clinical samples across the measuring range were assayed in replicates of two at two separate times per day for twenty days using a single lot of FREND™ PSA Plus cartridge. The findings follow showing repeatability, between-run, between-day and within-laboratory precision data.

Sample	Mean PSA	Repeatability		Between-run		Between-day		Within-laboratory	
	(ng/mL)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
1	0.098	0.013	12.80	0.005	5.5	0.004	3.7	0.014	14.4
2	4.321	0.248	5.7	0.054	1.2	0.089	2.1	0.269	6.2
3	12.735	0.636	5.0	0.405	3.2	0.102	0.8	0.761	6.0
4	25.462	1.278	5.0	0.668	2.6	0.321	1.3	1.477	5.8

2b) Precision Testing Multiple Lots and Multiple Sites

Three different lots of FREND™ PSA Plus were evaluated at three geographically diverse sites. Four replicates each of Material A, Material B, and Material C and two replicates of QC 1, QC 2 and QC 3 were evaluated in each of two runs performed for five days at each site. A total of 40 results on each material were generated at each of the three sites yielding a grand total of 120 replicates of each material. The data was analyzed using a CLSI format from EP5-A2 for an ANOVA analysis. Instrument-to-Instrument is the same as Site-to-Site in this scenario. As can be seen from the table below, the largest source of variation is the cartridge which would be the expected result. The FREND™ PSA Plus cartridge is a single use cartridge that contains all the reagents within the cartridge necessary to support the reactions.

%CV by Material						
	Material					
Variation Source	MAT A (0.29 ng/mL)	MAT B (3.67 ng/mL)	MAT C (18.33 ng/mL)	QC 1 (0.30 ng/mL)	QC 2 (2.93 ng/mL)	QC 3 (20.25 ng/mL)
Site-to-Site	3.50%	1.57%	1.67%	3.47%	1.61%	2.06%
Day-to-Day	0.00%	0.99%	1.21%	0.00%	0.00%	0.00%
Lot-to-Lot	9.12%	3.16%	7.01%	6.08%	4.30%	6.00%
Inter-cartridge	18.45%	6.81%	7.94%	20.03%	6.17%	7.49%
Total	20.87%	7.74%	10.79%	21.22%	7.69%	9.81%

3) Specificity

The following substances were evaluated for potential cross-reactivity with the FREND™ PSA Plus at the concentrations indicated below. Testing was done according to the instructions recommended by CLSI protocol EP7-A. No significant cross-reactivity was found.

Specificity of FREND™ PSA Plus

No.	Substrate	Concentration
1	PAP (Prostate Acid Phosphatase)	10.0 ng/mL
2	Kallikrein	15.0 ng/mL

4) Analytical Sensitivity

The Limit of Detection (LoD) for the FREND™ PSA Plus was determined using the CLSI EP17-A protocol. The analytical sensitivity of the FREND™ PSA Plus was determined to be 0.1 ng/mL.

16. Interference

Interference is defined, for purposes of this study, to be recovery outside of 15% of the known specimen mean concentration. In other words, recovery from 85% to 115% of the expected is considered acceptable performance.

1) Endogenous Interferents

These interference studies on endogenous substances were performed using the FREND™ PSA Plus on the FREND™ System according to the recommendations in the CLSI protocol EP7-A:

- Added hemoglobin (up to 500 mg/dL) does not interfere with the assay. Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 97.25%.
- Added unconjugated bilirubin (up to 20 mg/dL) does not interfere with the assay.
- Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 98.2%.
- Added gamma globulin (Total Protein) up to 5.0 g/dL does not interfere with the assay. Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 106.3%.
- Added triglyceride up to 3 g/dL does not interfere with this assay. Average recovery when added to serum containing tPSA at 1.0 and 4.0 ng/mL was 101.5%.

2) Pharmaceutical Interferents

The following chart shows the interference studies performed using the FREND™ PSA Plus on the FREND™ system for various drugs that might be found in the serum/plasma of men diagnosed with prostate cancer. The concentrations of the drugs that were added to the test samples and controls at base concentrations of tPSA of 1.0 ng/mL and 4.0 ng/mL are shown as are the % recoveries on the far right side of the chart. It is unlikely since the FREND™ PSA Plus method uses monoclonal antibodies that any substances without a tertiary structure similar to PSA would interfere. The testing showed that indeed there was no significant interference from the tested drugs that would affect the interpretation of a tPSA result as assayed on the FREND™ PSA Plus.

Interference Study Results for FREND™ PSA Plus on the FREND™ System

No.	Substrate	Concentration	Average Recovery %
1	Flutamide	10 µg/mL	94.50%
2	Diethylstilbestrol (DES)	5 µg/mL	103.80%
3	Goserelin	40 ng/mL	103.20%
4	Tamsulosin	100 ng/mL	98.85%
5	Acetaminophen	250 ng/mL	100.45%
6	Acetylsalicylic acid	600 µg/mL	95.85%
7	Leuprolide	275 ng/mL	101.50%
8	Ibuprofen	500 µg/mL	102.25%
9	Finasteride	250 ng/mL	93.60%
10	Docetaxel	10 µg/mL	114.45%

17. Serial Measurements and Concordance with Medical Status

Since the FREND™ PSA Plus Indication for Use is that the assay results will be used as a tool in managing the care for patients with prostate cancer, it is imperative that the changes in the marker are compared to clinical status changes to determine the efficacy of the test. Therefore, as an important part of the clinical studies performed to characterize the FREND™ PSA Plus, serial samples collected longitudinally from patients previously diagnosed with prostate cancer and treated in a variety of ways over the clinical course of their

disease (including prostatectomy, radioactive seeds, external beam radiation, chemotherapy, hormone therapy alone or in combination) were assayed for tPSA with the FREND™ PSA Plus on the FREND™ system. The same samples were also measured for tPSA by another FDA cleared method.

For each point to point in a sample serial set, the change in the tPSA concentration was compared to the change in the clinical status of the patients as measured by other laboratory tests, patient interviews, physical examinations, and imaging studies of a variety of types and recorded on a Clinical Report Form.

These changes in the tPSA marker concentration were defined as significant or not by multiplying the overall CV of the assay at the midrange (as determined by the test imprecision study) by a factor of 2.5 to define a percentage change different from what would be expected because of assay imprecision. For the FREND™ PSA Plus assay with an overall mid-range CV of 8.5%, significance was set at a change in excess of 20%. Any increase in value from one time period to the next that did not exceed 20% was logged as ≤ 20% Change. For the other FDA cleared method, significance was set at a change > 8.5%. This was calculated using that method's published overall mid-range CV of 3.4% x 2.5.

Physician's impressions regarding subject's disease status at each blood draw were recorded on CRFs for all serial PSA samples. The status information at one visit was compared with the status at the next and a change in status was determined for the visit pair. The change in clinical status from the physician's impression was then compared to significant changes in the PSA concentration of each sample as measured by both the test device (FREND™ PSA Plus) and the predicate device (FDA Cleared Assay). The results of these comparisons are shown below where clinical status was divided into two groups: those visit pairs showing progression and those showing no disease progression per the visit to visit status.

1) Samples with no Progression (Negative Concordance)

	Other FDA Cleared PSA Assay		
FREND™ PSA Plus	≥8.5%	<8.5%	Total
≥20%	31	15	46
<20%	15	67	82
Total	46	82	128

NC(FREND™ PSA Plus)	0.641
NC(AIA-PACK PSA)	0.641
Different	0.000

There is no difference in the Negative Concordance (NC) between the two assays.

2) Samples with Progression (Positive Concordance)

	Other FDA Cleared PSA Assay		
FREND™ PSA Plus	≥8.5%	<8.5%	Total
≥20%	83	1	84
<20%	5	19	24
Total	88	20	108

PC(FREND™ PSA Plus)	0.778
PC(AIA-PACK PSA)	0.815
Different	0.037

A McNemar test of the paired data in the above table yields a p value of 0.218. The associated 95% confidence interval for the true difference is - 0.0157 to 0.0551. Both estimators agree: there are no statistically significant differences in the Positive Concordances (PC) between the two assays.

Below is a chart comparing the concordances of the FREND™ PSA Plus assay and the other FDA cleared PSA assay. Based on the 95% confidence intervals there appears to be no differences between the concordances for the FREND™ PSA Plus assay and the other FDA cleared PSA assay.

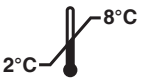
Concordance	FREND™ PSA Plus	95% CI*	FDA Cleared PSA	95% CI*
Positive	77.8%	69.8% to 85.3%	81.5%	73.8% to 88.7%
Negative	64.1%	55.1% to 71.5%	64.1%	55.1% to 71.5%
Total	70.3%	64.1% to 76.9%	72.0%	66.1% to 77.5%

* Confidence Intervals are based on 10,000 resamples of the patient data.

18. References

1) de Koning HJ, Liem MK, Baan CA, Boer R, Schröder FH, Alexander FE, Prostate cancer mortality reduction by screening: power and time frame with complete enrollment in the European Randomized Screening for Prostate Cancer (ERSPC) trial. *International Journal Cancer*, 2002; 98:268-73.
2) Wan-Ming Zhang, Patrik Finne, Jari Leinonen et al., Characterization and immunological determination of the complex between prostate-specific antigen and a2-macroglobulin, *Clinical Chemistry*, 1998, 44:12, 2471–2479.
3) David L. Woodrum et al., Analytical performance of the Tandem-R free PSA immunoassay measuring free prostate-specific antigen. *Clinical Chemistry*, 1997, 43:7, 1203–1208.
4) Yun Sik Kwak, M.D., Appropriate use of Prostate-Specific Antigen in Diagnosing Carcinoma of the Prostate. *Journal Korean Geriatrics Society*, 2009, Vol.13, Mar, pp. 12-17.
5) Chen Z, Prestigiacomo A, et al., Purification and characterization of prostate-specific antigen(PSA) complexed to alpha 1-antichymotrypsin: potential reference material for international standardization of PSA immunoassays, *Clinical Chemistry*, 1995, 41(9):1273-82.
6) Wang, M.C., et al, 1981, Prostatic Antigen: A New Potential Marker for Prostatic Cancer. *Prostate* 2:89.
7) Frankel, A.G., et al, 1982, Monoclonal Antibodies to a Human Prostate Antigen. *Cancer Res*. 42:3714
8) Papsidero, L.D., et al, 1980, A Prostate Antigen in Sera of Prostatic Cancer Patients. *Cancer Res.* 40:2428.
9) Kuriyama, M., et al, 1980, Quantitation of Prostate-Specific Antigen in Serum by a Sensitive Enzyme Immunoassay. *Cancer Res.* 40:4658.
10) Killian, C.S., 1985, Prognostic Importance of Prostate-Specific Antigen for Monitoring Patients with Stages B2 to D1 Prostate Cancer. *Cancer Res*. 45:886.
11) Kuriyama, M., et al, 1982, Multiple Marker Evaluation in Human Prostate Cancer with the Use of Tissue-Specific Antigens. *J. Nat. Canc*. 68:99.
12) Nahm MH and Goffman JW. Heteroantibody: Phantom of the Immunoassay. *Clinical Chemistry*, 1990, 36:829.
13) Boscato LM., Stuart MC., Heterophilic Antibodies: A Problem for All Immunoassay Compared. *Clinical Chemistry,* 1988, 33:1916-20.
14) Young, D., 1990, Effects of Drugs on Clinical Laboratory Tests. 3rd Edition, Washington, DC, *American Association for Clinical Chemistry* Press.
15) Chan DW, Bruzek DJ,Oesterling JE, et al., Human Anti-Murine Immunoassays, *Clinical Chemistry,* 1997, 1916-20.

19. Glossary of Symbols

	Do not reuse
	Use by YYYY-MM-DD
	Lot number
	Catalog number
	Warning or Caution
	Manufactured by
	Authorized representative in the Europe Community
	In vitro diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests

NanoEnTek

sales@nanoentek.com
www.nanoentekinc.com

Manufactured by :

NanoEnTek Inc. (HQ)
12F, 5, Digital-ro 26-gil, Guro-gu, Seoul, 152-740, Korea
Tel : +82-2-6220-7940 Fax : +82-2-6220-7721

US Branch

NanoEnTek USA Inc.
5627 Stoneridge Drive Suite 304, Pleasanton, CA 94588, USA
Tel : +1-925-225-0108, +1-888-988-0108 (Toll free) Fax : +1-925-225-0109

EC REP

MT Promedt Consulting GmbH
Altenhofstrasse 80, 66386 St. Ingbert, Germany

SK Medical (Beijing) Co., Ltd.
26F SK tower No.6 Jia, Jianguomenwai Avenue, Chaoyang District, Beijing,
100022 P.R. China Tel : +86-10-5920-7844 Fax : +86-10-5920-5697